HORMONAL MODULATION OF MUSCLE ANDROGEN RECEPTORS: CYTOSOLIC AND TOTAL BINDING

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ABSTRACT

The androgen receptor in rat striated muscle (levator ani muscle and skeletal muscle) was quantified in homogenate and cytosol fractions in order to relate receptor concentrations and subcellular distribution to sex differences, maturity, and effects imposed by castration. Muscle cytosolic androgen receptor binding was elevated following orchiectomy. Muscle homogenate receptor concentrations of orchiectomized male rats also were elevated (165% control, levator ani muscle; 132% control, skeletal muscle) relative to intact male values but not to the same degree as cytosolic levels (500% control, levator ani muscle; 176% control, skeletal muscle). Homogenate receptor levels in skeletal muscle from immature male or adult female rats were not elevated in comparison to adult male values even though their cytosolic receptor levels were appreciably higher. Androgen receptor apparent subcellular distribution varied: adult male rats had the lowest percentage of receptors in the cytosol fraction (40%), whereas adult females had the highest percentage of receptors in the cytosol fraction (87%). These results support the hypothesis of androgen receptor down-regulation by testosterone, and they indicate further that cytosolic receptor levels do not necessarily reflect muscle sensitivity to androgens.

INTRODUCTION

Androgens have marked effects on the metabolism of striated muscle (1, 2, 3, 4). Because these actions appear to be mediated via intracellular receptors (5, 6, 7), the concentration of such receptors may be a determinant of the androgen sensitivity of

muscle (8). Reports from this laboratory (10) and from that of Dahlberg et al.(9) suggest that muscle androgen receptors are down-regulated by endogenous androgens. The concentration of cytosolic receptors increases dramatically following orchiectomy (9, 10), and this increase appears to be correlated with enhanced ability of testosterone to induce glucose 6-phosphate dehydrogenase in the levator ani muscle (30). Furthermore, the cytosolic androgen receptor concentration is higher in muscle from immature and orchiectomized male rats, as well as from female rats, than in muscle from adult male rats (9). Administration of testosterone propionate reversed the increase in cytosolic receptor bindng in skeletal muscle following orchiectomy (10). Testosterone propionate may decrease cytosolic receptor levels by down-regulating receptor synthesis, by filling receptor binding sites, or by increasing high affinity nuclear binding and thereby lowering the cytosolic fraction. To address this issue, we measured total muscle (i.e., homogenate) androgen receptor binding in immature, adult, and orchiectomized male rats and also in adult female rats. We compared total muscle receptor binding with cytosolic receptor binding. The results to be described reveal that total muscle androgen receptors of orchiectomized male rats but not immature male or adult female rats are elevated relative to adult males. Adult male rats have the lowest proportion of androgen receptors in the cytosol fraction in comparison with orchiectomized or immature males or adult females.

EXPERIMENTAL

Animals - Rats of both sexes of the Crl:CD(SD)BR strain (Charles River Breeding Labs, Wilmington, MA) were used. They were housed in a 12 h lights on:12 h lights off cycle, and they were fed Purina Rodent Laboratory Chow (#5001) and water ad libitum. Adult males and females weighed 150-200 g; immature (24 days old) rats weighed 40-50 g. Orchiectomy was performed via the abdominal route under ether anesthesia. The muscles used in this study were the hormone-dependent levator ani muscle and "ordinary" skeletal muscle (tibialis anterior plus extensor digitorum longus).

Androgen Receptor Binding - This was assessed using [^{3}H] methyltrienolone (17β-hydroxy-17α-methyl-4,9,11-estratrien-3-one; R1881; specific activity 86 mCi/mmol/New England Nuclear, Waltham, MA). We measured total muscle (i.e., homogenate) and cytosolic androgen receptor binding as described (11). Briefly, minced muscle was homogenized in a Polytron homogenizer in ice-cold buffer (5 ml/g muscle) comprising 25 mM Na_2HPO_4, 1.5 mM EDTA, 10% (v/v) glycerol, 2 mM dithiothreitol, 10 mM, sodium molybdate, and 1 mM phenylmethylsulfonyl fluoride, pH 7.4. The homogenate was filtered through a 40 mesh Nitex screen (Tetko, Inc., Elmsford, NY) to remove connective tissue and other debris. Cytosol was prepared by centrifugation of the homogenate at 110,000 x g for 1 h at 0-4°C in a Sorvall OTD centrifuge. Homogenate and cytosol binding were performed both with single-point assays (15 nM [^{3}H] -methyltrienolone) and by Scatchard analysis (12). Triamcinolone acetonide was included in all incubations to prevent binding to

progestin and glucocorticoid receptors (13, 14). After incubation at 0-4°C for 24 h free and bound ligand were separated using a filtration assay employing hydroxylapatite (Bio-Gel HTP, Biorad, Richmond, CA) as described (15). [^{3}H] methyltrienolone binding in muscle homogenate was maximal after incubation for 24 h at 0-4° (11). Moreover, 80% exchange occurred at 24 h at 0-4° when occupied muscle androgen receptor (prepared from a muscle homogenate from a castrated rat incubated with 5 nM [^{3}H] 5α -dihydrotestosterone for 4 h) was incubated with 15 nM methyltrienolone and diluted four-fold (11).

Protein was determined according to Lowry et al. (16).

DNA was determined as described (17) using calf thymus DNA as standard.

Chemicals - Unless otherwise stated, all chemicals were from Sigma Chemical Co. (St. Louis, Mo.).

Data Analysis - Statistical analysis was performd with a t-test. Scatchard plot data were computed with the program of McPhersen (18), using an IBM Personal Computer. Ninety-five percent confidence intervals for the ratios in Fig. 4 were determined using Fieller's Theorem (28).

RESULTS

Levator Ani Muscle

Using saturating levels of [^{3}H] methyltrienolone, apparent maximal methyltrienolone binding in levator ani muscle cytosol increased significantly to 500% of the control value 14 days after orchiectomy (GDX) (Fig. 1; 10). The magnitude of the increase in levator ani muscle homogenate following orchiectomy was less

(165%) control (Fig. 1), albeit significant. Binding affinities (apparent K_D) determined from Scatchard analysis of the cytosolic and homogenate levator ani muscle preparations were similar (0.68 and 1.12 nM, respectively) and were not significantly changed following orchiectomy.

Skeletal Muscle

We then analyzed specific binding of [^{3}H] methyltrienolone to cytosol and homogenate preparations from skeletal muscle from immature, adult, and 14-day post-orchiectomy male rats and adult female rats. The apparent K_D of both the cytosol and homogenate skeletal muscle preparations was determined from Scatchard analysis, and it was somewhat smaller in all animal groups (Table 1).

Using saturating levels of [^{3}H] methyltrienolone, apparent maximal methyltrienolone binding in skeletal muscle homogenate preparations was determined in each of the animal groups. On a gram wet weight basis, maximal [^{3}H] methyltrienolone binding in muscle from adult males did not differ significantly from that from immature or 14 day post-orchiectomy males (Fig. 2). However, comparison of [^{3}H] methyltrienolone binding on a mg DNA basis revealed significant differences among the animal groups. Muscle from immature male rats had significantly lower muscle receptor concentrations/mg DNA (43%) whereas 14 day post-GDX males had significantly higher levels (132%). This was due to higher levels of DNA/g wet weight in immature male rats ($p < 0.05$) and lower values in 14 day post orchiectomy male rats ($p < 0.05$) than gonadally intact adult male rats.

Maximal [^{3}H] methyltrienolone binding in muscle homogenate from adult female rats was significantly lower (69%) than adult male values on a g wet weight basis (Fig. 2). However, adult female rats had lower DNA content/g wet weight ($p < .05$) muscle than adult male rats, and maximal methyltrienolone binding in skeletal muscle homogenate from adult females was not significantly different on a mg DNA basis from adult male values (Fig. 2).

As expected (9), cytosolic muscle androgen receptor concentrations from adult males are lower than those of adult female or immature and 14 day post-orchiectomy male rats either on a g wet weight or a mg protein basis (Fig. 3). Low cytosolic receptor levels in skeletal muscle of adult male rats (Fig. 3) reflects a different distribution of total cellular androgen receptors (40% cytosol, Fig. 4). Adult female rats and 14 day post-orchiectomy rats had a high percentage of the total receptors localized to the cytosol; i.e., % of total value (87% and 78%, respectively) and immature males had an intermediate distribution (64%). This approach (i.e., comparison of total homogenate and cytosolic androgen receptor binding) is validated by the crude muscle fractionation study of Table II. Earlier work showed that such fractionation results in 99 ± 14% recovery of total homogenate receptors (11); other fractions were: 1000 x g pellet, 61 ± 14%; 20,000 x g supernate, 36 ± 7%; 20,000 x g pellet, 4 ± 1% (11). Table II shows a remarkable shift in this distribution in muscle from castrated rats, with almost all of the receptor recoverable in the cytosol fraction. Given the error inherent in

such measurements in crude tissue fractions, this result is in reasonable agreement with that of Fig. 4.

DISCUSSION

Receptor localized to the cytosol fraction of muscle homogenate may be either the cytoplasmic receptor in the classical "two-step" model of steroid hormone action (20, 21) or, alternatively, it may reside in the nucleus in vivo, in which case low affinity association has been disrupted by tissue homogenization (22, 23, 29). We measured androgen receptors in muscle homogenates along with the cytosolic fraction to discriminate between altered receptor synthesis and a change in intracellular distribution or nuclear affinity (11). Presumably, the difference between total and cytosolic binding represents mainly receptor bound to high affinity nuclear acceptor sites. However, a small fraction may represent high affinity binding to plasma membranes (24, 25). Since androgen-specific alteration of gene transcription and expression is considered to involve nuclear binding, the measurement of nuclear receptor levels in target tissues is desirable. However, it has not been possible to isolate nuclear material from skeletal muscle with acceptable recovery (19). Studies on steroid hormone receptors in muscle have, therefore, relied on quantification of receptor in the cytosolic fraction.

In the present study we measured total androgen receptors in rat muscle in order to relate receptor quantitites to sex differences, maturity, and effects imposed by castration. For the levator ani muscle, homogenate binding was significantly elevated

14 days after orchiectomy, although the increase was smaller than that seen in the cytosol fraction (Fig. 1). This result, with the cycloheximide-sensitivity of the orchiectomy effect (10), suggests that the increase in androgen receptor binding following orchiectomy results, in part, from de novo receptor synthesis. Clearly, however, a shift in the intracellular distribution of the receptor also occurred.

For skeletal muscle (extensor digitorum longus and tibialis anterior), homogenate binding affinities were consistently higher than cytosolic values in all animal groups (Table 1). Dahlberg et al. (9) reported that skeletal muscle androgen receptor K_D increases significantly with age in the rat and is decreased in females or following castration in males. We found a similar trend, but the magnitude of differences in binding affinities are smaller than those reported by Dahlberg et al. (9).

Total androgen receptors from skeletal muscle of orchiectomized male rats were elevated relative to adult male values (Fig. 2). However, total receptor levels in muscle from immature males or adult females were not elevated in comparison to adult male values (Fig. 2), even though their cytosolic receptor levels were (Fig. 3). Thus, whether testosterone down-regulates the androgen receptor in skeletal muscle, as it apparently does in levator ani muscle, is unclear.

Adult male rats have the lowest percentage of total androgen receptors in the cytosol fraction in comparison with orchiectomized or immature males, or adult females (Fig. 4). This observation suggests that adult male rats have the highest levels

of androgen receptors in the nucleus. Seventy-eight percent of skeletal muscle androgen receptors are localized to the cytosol 14 days post orchiectomy; the remainder presumably are bound to nuclear elements. Possibly, adrenal cortical synthesis of androgenic hormones is sufficient to fill some receptor sites. Alternatively or additionally, the remaining receptors may be unoccupied receptors bound to nuclear elements with high affinity. In this regard, several reports have demonstrated unfilled estrogen receptors in the nucleus of estrogen target tissues (26, 27). Thus, measurement of cytosolic receptors alone may not necessarily reflect steroid hormone sensitivity of muscle.

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Table 1

Androgen Receptor Binding Affinities (K_D) of Tibialis Anterior and Extensor Digitorum Longus Muscles

Animal Group	K_D (nM)	
	Cytosol	Homogenate
Adult males (200 g)	0.72	1.18
Adult females (200 g)	0.38	0.86
Immature males (45 g)	0.49	1.13
Orchiectomized males (200 g)	0.43	0.63

The orchiectomized males were killed 14 d after the operation. The number in parentheses is body weight. Other experimental details are described in the text.

Table II

Subcellular Fractionation of Skeletal Muscles from Control and Orchiectomized Adult Male Rats

Fraction	% Total Binding	
	Ctl	GDX
Homogenate	100	100
1,000 x g pellet	51	0
20,000 x g supernate	46	95
20, 000 x g pellet	3	5

Orchiectomized rats were killed 14 days after the operation. The data are means of 2 determinations.

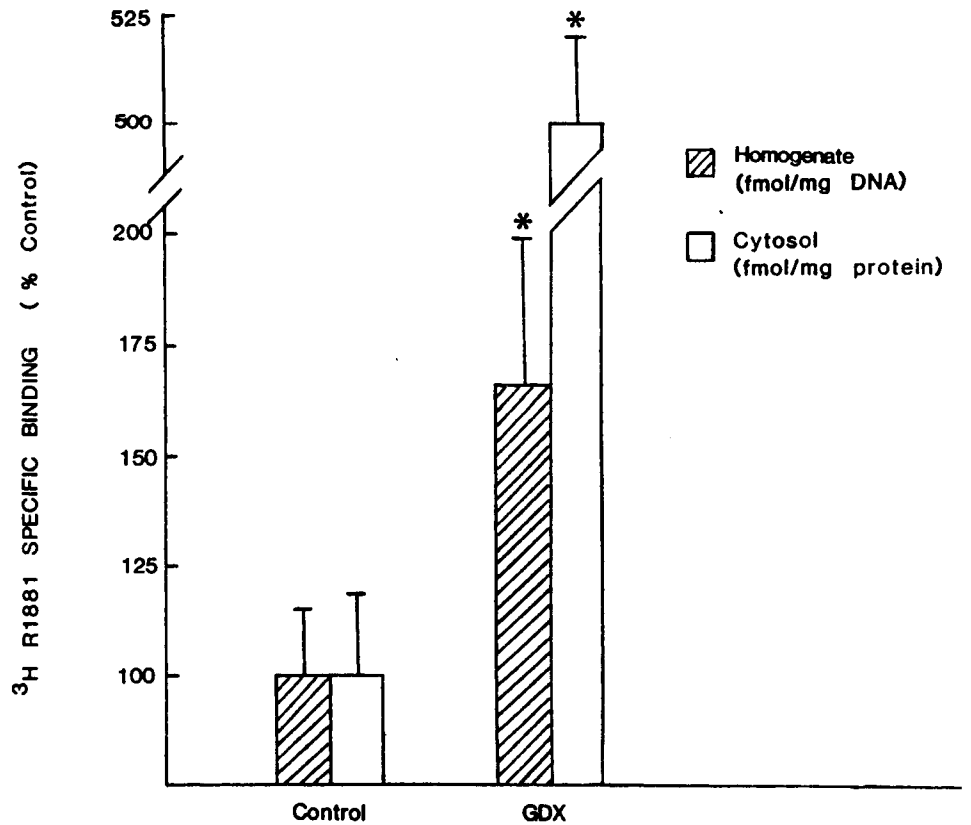
FIGURE LEGENDS

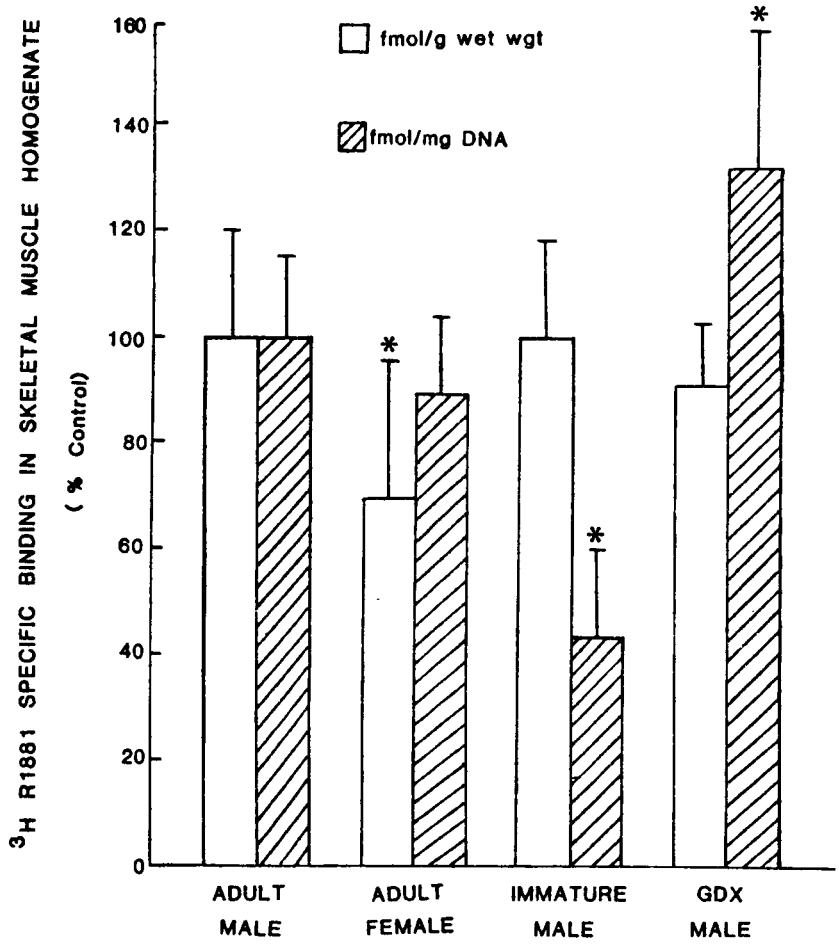
Figure 1 - Effect of orchiectomy on [^{3}H] methyltrienolone specific binding in rat levator ani muscle homogenate and cytosol. Rats were sacrificed 14 days post-orchiectomy. Data are means ± S.D. of 4 (homogenate) or 6 (cytosol) separate determinations. GDX = orchiectomy.

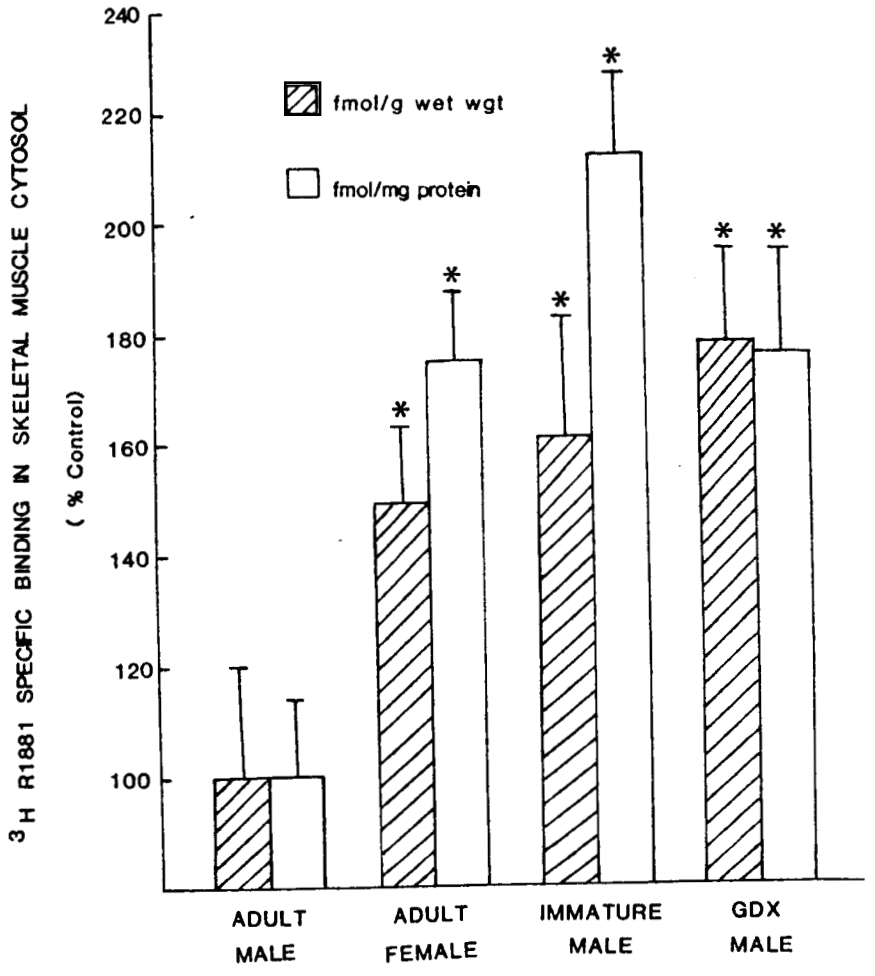
Figure 2 - [^{3}H] methyltrienolone specific binding in skeletal muscle (tibialis anterior and extensor digitorum longus muscles) homogenates of male (adult, immature, and orchiectomized) and adult female rats. Orchiectomized rats were killed 14 days after the operation. Androgen receptor binding was performed with single-point assays (15 nM methyltrienolone). Values are means ± S.D. In adult rats, 5 or more animals were analyzed individually. For the case of immature male rats, skeletal muscle tissue was pooled from 5-7 animals/determination to obtain 4 separate determinations. *Significantly different from the control (i.e., adult male) value, $P < 0.05$. GDX = orchiectomy.

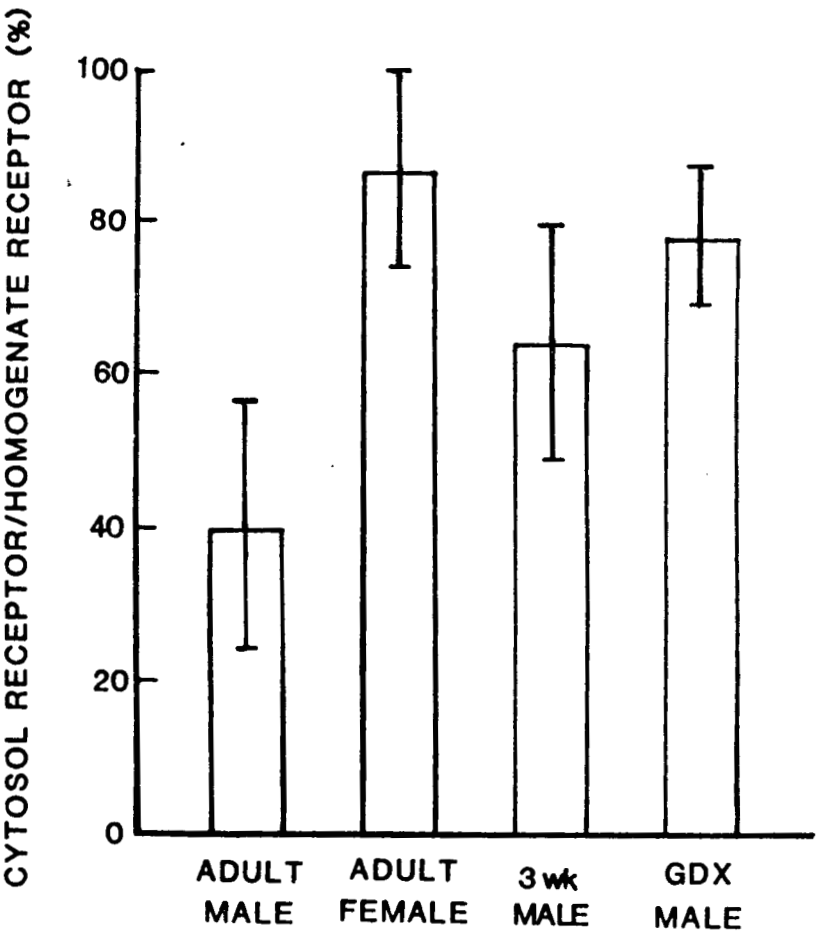
Figure 3 - [^{3}H] methyltrienolone specific binding in skeletal muscle (tibialis anterior and extensor digitorum longus muscles) cytosols of male (adult, immature and orchiectomized) and adult female rats. Orchiectomized rats were killed 14 days after the operation. Androgen receptor binding was performed with single-point assays (15 nM methyltrienolone). Values are means ± S.D. In adult rats, 9 or more animals were individually analyzed. For the case of immature male rats, skeletal muscle tissue was pooled from 5-7 animals/determination to obtain 9 separate determinations. *Significantly different from the control (i.e., adult male) value, $P < 0.05$). GDX = orchiectomy.

Figure 4 - Distribution of skeletal muscle androgen receptors. The percentage of total (homogenate) receptor detected in the cytosolic fraction of each animal group is shown. Data for homogenate values and cytosolic values are taken from Figs. 2 and 3 respectively (per g wet wt basis). Values are means ± 95% confidence intervals. GDX = orchiectomy.


525
500
200
175
150
125
100
^{3}H R1881 SPECIFIC BINDING (% Control)
*
*
Homogenate
(fmol/mg DNA)
Cytosol
(fmol/mg protein)
Control
GDX


^{3}H R1881 SPECIFIC BINDING IN SKELETAL MUSCLE HOMOGENATE
(% Control)
fmol/g wet wgt
fmol/mg DNA
160
140
120
100
80
60
40
20
0
*
*
*
ADULT MALE
ADULT FEMALE
IMMATURE MALE
GDX MALE


^{3}H R1881 SPECIFIC BINDING IN SKELETAL MUSCLE CYTOSOL
(% Control)
240
220
200
180
160
140
120
100
fmol/g wet wgt
fmol/mg protein
*
ADULT MALE
ADULT FEMALE
IMMATURE MALE
GDX MALE


CYTOSOL RECEPTOR/HOMOGENATE RECEPTOR (%)
100
80
60
40
20
0
ADULT MALE
ADULT FEMALE
3 wk MALE
GDX MALE